Exercise 7

# Enrichment analysis

## Load Libraries

library(rGREAT)  
library(TxDb.Mmusculus.UCSC.mm10.knownGene)  
library(org.Mm.eg.db)  
library(simplifyEnrichment)  
library(flexclust)

## OverlapMatrix

overlap <- readRDS("output/overlap\_anno2.rds")

## Running enrichment analysis

gr <- overlap[overlap$ATAC\_RNA == "Active Promoters"]  
res <- great(gr, "BP", "mm10")

\* TSS source: TxDb.

\* check whether TxDb package 'TxDb.Mmusculus.UCSC.mm10.knownGene' is installed.

\* gene ID type in the extended TSS is 'Entrez Gene ID'.

\* restrict chromosomes to 'chr1, chr2, chr3, chr4, chr5, chr6, chr7, chr8, chr9, chr10,  
 chr11, chr12, chr13, chr14, chr15, chr16, chr17, chr18, chr19, chrX, chrY, chrM'.

\* 20593/24515 protein-coding genes left.

\* update seqinfo to the selected chromosomes.

\* TSS extension mode is 'basalPlusExt'.

\* construct the basal domains by extending 5000bp to upstream and 1000bp to downsteram of TSS.

\* calculate distances to neighbour regions.

\* extend to both sides until reaching the neighbour genes or to the maximal extension.

\* use GO:BP ontology with 15445 gene sets (source: org.Mm.eg.db).

\* check gene ID type in `gene\_sets` and in `extended\_tss`.

\* use whole genome as background.

\* remove excluded regions from background.

\* overlap `gr` to background regions (based on midpoint).

\* in total 99 `gr`.

\* overlap extended TSS to background regions.

\* check which genes are in the gene sets.

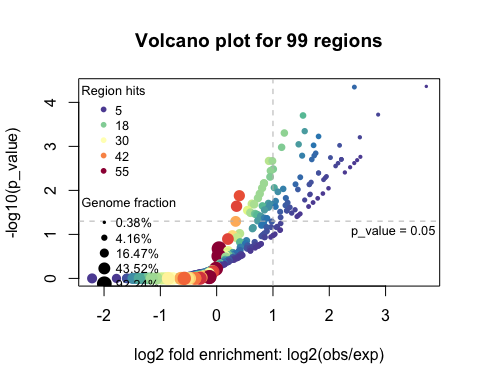
\* only take gene sets with size >= 5.

\* in total 9412 gene sets.

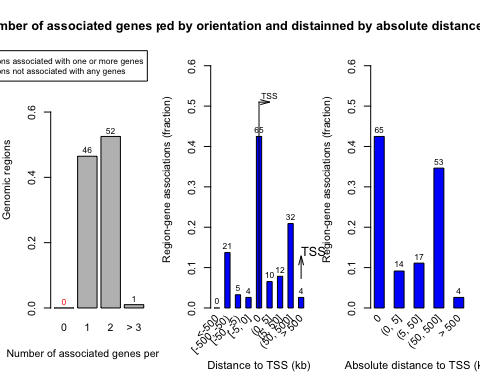
\* overlap `gr` to every extended TSS.

\* perform binomial test for each biological term.

plotVolcano(res)



plotRegionGeneAssociations(res)



getRegionGeneAssociations(res)

GRanges object with 99 ranges and 112 metadata columns:  
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 peak\_69 chr1 25228208-25229714 \* | Promoter (<=1kb)  
 peak\_70 chr1 25504015-25505076 \* | Exon (ENSMUST0000014..  
 peak\_83 chr1 34578812-34580363 \* | Promoter (<=1kb)  
 ... ... ... ... . ...  
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 peak\_1922 chr2 181134082-181135787 \* | Promoter (<=1kb)  
 peak\_1923 chr2 181156149-181157204 \* | Promoter (<=1kb)  
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 peak\_69 1 25067476 25228826 161351  
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 peak\_69 2 210933 ENSMUST00000126626.7  
 peak\_70 2 100504323 ENSMUST00000193138.1  
 peak\_83 1 211383 ENSMUST00000052670.10  
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 peak\_1922 2 16536 ENSMUST00000149964.8  
 peak\_1923 2 13628 ENSMUST00000055990.7  
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 peak\_70 -226061 ENSMUSG00000104257 Gm20172  
 peak\_83 0 ENSMUSG00000045174 Amer3  
 ... ... ... ...  
 peak\_1902 0 ENSMUSG00000027525 Phactr3  
 peak\_1922 0 ENSMUSG00000016346 Kcnq2  
 peak\_1923 0 ENSMUSG00000016349 Eef1a2  
 peak\_1925 0 ENSMUSG00000027581 Stmn3  
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 peak\_69 adhesion G protein-c.. Promoter 0.986539 8.25070 40.79772  
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 peak\_1925 1.54857e-06 7.67150e-06 ENSMUSG00000027581 20262 Stmn3  
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 peak\_83 chr1 34579657 34590944 + 211383  
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 peak\_69 Adgrb3 adhesion G protein-c.. Promoter 1.71288  
 peak\_70 Gm20172 predicted gene, 20172 Exon 2.45135  
 peak\_83 Amer3 APC membrane recruit.. Promoter 1.44331  
 ... ... ... ... ...  
 peak\_1902 Phactr3 phosphatase and acti.. Promoter 1.382198  
 peak\_1922 Kcnq2 potassium voltage-ga.. Promoter 0.909119  
 peak\_1923 Eef1a2 eukaryotic translati.. Promoter 1.426917  
 peak\_1925 Stmn3 stathmin-like 3 Promoter 1.337649  
 peak\_1930 Oprl1 opioid receptor-like 1 Promoter 1.363849  
 H3K27ac\_logCPM H3K27ac\_F H3K27ac\_PValue H3K27ac\_qvalue name  
 <numeric> <numeric> <numeric> <numeric> <character>  
 peak\_1 8.81735 151.4959 1.67395e-34 4.08130e-32 peak\_1  
 peak\_14 7.44136 188.6314 1.91288e-42 6.62156e-40 peak\_14  
 peak\_69 7.75798 148.3754 7.82086e-34 1.80483e-31 peak\_69  
 peak\_70 5.04358 34.5321 4.36085e-09 1.34796e-07 peak\_70  
 peak\_83 7.35920 86.0818 2.18441e-20 2.14293e-18 peak\_83  
 ... ... ... ... ... ...  
 peak\_1902 6.22622 35.9816 2.07855e-09 6.74235e-08 peak\_1902  
 peak\_1922 8.31643 64.4153 1.15104e-15 7.72601e-14 peak\_1922  
 peak\_1923 7.39512 86.2073 2.05151e-20 2.03730e-18 peak\_1923  
 peak\_1925 8.00741 111.4177 7.08864e-26 1.02853e-23 peak\_1925  
 peak\_1930 7.84616 104.9298 1.79151e-24 2.34334e-22 peak\_1930  
 ATAC\_RNA annotated\_genes dist\_to\_TSS  
 <character> <CharacterList> <IntegerList>  
 peak\_1 Active Promoters Xkr4 0  
 peak\_14 Active Promoters Sntg1 0  
 peak\_69 Active Promoters Lmbrd1,Gm20172 549578,48240  
 peak\_70 Active Promoters Gm20172,Adgrb3 -226061,324631  
 peak\_83 Active Promoters Amer3 0  
 ... ... ... ...  
 peak\_1902 Active Promoters Phactr3,Sycp2 56194,231798  
 peak\_1922 Active Promoters Chrna4,Kcnq2 -90536,0  
 peak\_1923 Active Promoters Eef1a2 0  
 peak\_1925 Active Promoters Gmeb2,Stmn3,Rtel1 -25237,0,-4903  
 peak\_1930 Active Promoters Oprl1 0  
 -------  
 seqinfo: 21 sequences from an unspecified genome; no seqlengths

shinyReport(res)

tb = getEnrichmentTable(res)  
sig\_go\_ids = tb$id[tb$p\_adjust < 0.05]  
cl = simplifyGO(mat = sig\_go\_ids)

You haven't provided value for `ont`, guess it as `BP`.

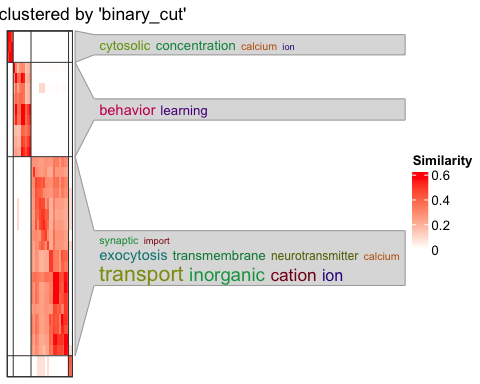
relations: is\_a, part\_of, regulates, negatively\_regulates, positively\_regulates

IC\_method: IC\_annotation

term\_sim\_method: Sim\_XGraSM\_2013

IC\_method: IC\_annotation

Cluster 33 terms by 'binary\_cut'... 4 clusters, used 0.111398 secs.  
'magick' package is suggested to install to give better rasterization.  
  
Set `ht\_opt$message = FALSE` to turn off this message.  
Perform keywords enrichment for 4 GO lists...



### Output

|  |
| --- |
| Important |
| * The heatmap shows clear clustering of GO terms into two main clusters, indicating distinct functional groups. * The GO terms on the right highlight biological processes relevant to neuronal and ion transport functions, such as transport, inorganic cation ion, exocytosis, synaptic, neurotransmitter, and behavior learning. * The cluster with terms like cytosolic concentration, calcium ion suggests a calcium-related signaling or ion homeostasis process at play. * The color gradient and similarity scores (up to ~0.6) indicate moderate similarity within clusters, which is reasonable for GO term groupings. |

### Interpretation

|  |
| --- |
| Important |
| * E11.5 to E15.5 is a key window in neural development involving neuronal differentiation, synapse formation, and active signaling. * Active promoters linked to ion transport, synaptic transmission, calcium signaling are expected to be regulated during this developmental stage. * The clustering separating signaling and transport-related terms from behavior and learning terms also aligns with early neural circuit formation and functional maturation. |

## Question

**Perform enrichment analysis of any other category.**